Evaluation of dietary treatment and amino acid supplementation in organic acidurias and urea-cycle disorders: On the basis of information from a European multicenter registry

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Abstract: Organic acidurias (OAD) and urea-cycle disorders (UCD) are rare inherited disorders affecting amino acid and protein metabolism. As dietary practice varies widely, we assessed their long-term prescribed dietary treatment against published guideline and studied plasma amino acids levels. We analyzed data from the first visit recorded in the European registry and network for intoxication type metabolic diseases (E-IMD, Chafea no. 2010 12 01). In total, 271 methylmalonic aciduria (MMA) and propionic aciduria (PA) and 361 UCD patients were included. Median natural protein prescription was consistent with the recommended daily allowance (RDA), plasma L-valine (57%), and L-isoleucine (55%) levels in MMA and PA lay below reference ranges. Plasma levels were particularly low in patients who received amino acid mixtures (AAMs-OAD) and L-isoleucine:L-leucine:L-valine (BCAA) ratio was 1.0:3.0:3.2. In UCD patients, plasma L-valine, L-isoleucine, and L-leucine levels lay below reference ranges in 18%, 30%, and 31%, respectively. In symptomatic UCD patients who received AAM-UCD, the median natural protein prescription lay below RDA, while their L-valine and L-isoleucine levels and plasma BCAA ratios were comparable to those in patients who did not receive AAM-UCD. Notably, in patients with ornithine transcarbamylase syndrome (OTC-D), carbamylphosphate synthetase 1 syndrome (CPS1-D) and hyperammonemia-hyperornithinemia-homocitrullinemia (HHH) syndrome selective L-citrulline supplementation resulted in higher plasma L-arginine levels than selective L-arginine supplementation. In conclusion, while MMA and PA patients who received AAMs-OAD had very low BCAA levels and disturbed plasma BCAA ratios, AAMs-UCD seemed to help UCD patients obtain normal BCAA levels. In patients with OTC-D, CPS1-D, and HHH syndrome, selective L-citrulline seemed preferable to selective L-arginine supplementation.

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ORIGINAL ARTICLE

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Evaluation of dietary treatment and amino acid supplementation in organic acidurias and urea-cycle disorders: On the basis of information from a European multicenter registry

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Organic acidurias (OAD) and urea-cycle disorders (UCD) are rare inherited disorders affecting amino acid and protein metabolism. As dietary practice varies widely, we assessed their long-term prescribed dietary treatment against published guideline and studied plasma amino acids levels. We analyzed data from the first visit recorded in the European registry and network for intoxication type metabolic diseases (E-IMD, Chafea no. 2010 12 01). In total, 271 methylmalonic aciduria (MMA) and propionic aciduria (PA) and 361 UCD patients were included. Median natural protein prescription was consistent with the recommended daily allowance (RDA), plasma L-valine (57%), and L-isoleucine (55%) levels in MMA and PA lay below reference ranges. Plasma levels were particularly low in patients who received amino acid mixtures (AAMs-OAD) and L-isoleucine:L-leucine:L-valine (BCAA) ratio was 1.0:3.0:3.2. In UCD patients, plasma L-valine, L-isoleucine, and L-leucine levels lay below reference ranges in 18%, 30%, and 31%, respectively. In symptomatic UCD patients who received AAM-UCD, the median natural protein prescription lay below RDA, while their L-valine and L-isoleucine levels and plasma BCAA ratios were comparable to those in patients who did not receive AAM-UCD. Notably, in patients with ornithine transcarbamylase syndrome (OTC-D), carbamylphosphate synthetase 1 syndrome (CPS1-D) and hyperammonemia-hyperornithinemia-homocitrullinemia (HHH) syndrome selective L-citrulline supplementation resulted in higher plasma L-arginine levels than selective L-arginine supplementation. In conclusion, while MMA and PA patients who received AAMs-OAD had very low BCAA levels and disturbed plasma

Abbreviations: AAM(s), amino acid mixture(s); AAM(s)-OAD, amino acid mixture(s) for organic acidemias (lack L-isoleucine and L-valine); AAM(s)-UCD, amino acid mixture(s) for urea-cycle disorders (contain essential amino acids); ASL(-D), argininosuccinate lyase (deficiency); ASS(-D), argininosuccinate synthetase (deficiency); BCAAs, branched-chain amino acids; CPS1(-D), carbamylphosphate synthetase 1 (deficiency); E-IMD, European registry and network for intoxication type metabolic diseases; HHH, hyperornithinemia-hyperammonemia-homocitrullinuria; MMA, methylmalonic aciduria; OAD, organic acidurias; OTC (-D), ornithine transcarbamylase (deficiency); PA, propionic aciduria; RDA, recommended daily allowance; SAA, single amino acids (L-valine and/or L-isoleucine supplied as a supplement, either independently or in combination); UCD, urea-cycle disorders; WHO, World Health Organization; Ws, Wilcoxon rank sum test.

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BCAA ratios, AAMs-UCD seemed to help UCD patients obtain normal BCAA levels. In patients with OTC-D, CPS1-D, and HHH syndrome, selective L-citrulline seemed preferable to selective L-arginine supplementation.

KEYWORDS
amino acid mixtures, branched-chain amino acids, dietary and supplemental treatment, L-citrulline and L-arginine, organic acidurias, urea-cycle disorders

1 | INTRODUCTION

Organic acidurias (OAD) and urea-cycle disorders (UCD) are rare inherited disorders affecting amino acid and protein metabolism. Their estimated incidence is 1 in 1 000 000 to 50 000 newborns per individual disease.[1,2] OAD (methylmalonic aciduria [MMA; mutase deficiency OMIM #251000, CblA #251100, CblB #251110] and propionic aciduria [PA; OMIM #606054]) are caused by deficiencies of the enzymes needed for the breakdown of branched-chain amino acids (BCAA), L-valine, and L-isoleucine in MMA and PA. UCD, that is, inherited deficiency of N-acetylglutamate synthase (OMIM #237310), carbamylphosphate synthetase 1 deficiency (CPS1-D) (EC 6.3.4.16; OMIM #237300), ornithine transcarbamylase deficiency (OTC-D) (EC 2.1.3.3; OMIM #311250), argininosuccinate synthetase deficiency (ASS-D) (EC 6.3.4.5; OMIM #215700), argininosuccinate lyase deficiency (ASL-D) (EC 4.3.2.1; OMIM #207900), arginase 1 deficiency (OMIM #207800), and hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome (OMIM #238970), are caused by deficiencies of the enzymes and transporter needed for the urea-cycle. If left untreated, ammonium and other toxic metabolites accumulate.

In OAD patients, long-term treatment consists of reducing natural protein intake to prevent intoxication while allowing normal growth.[3] Although, when necessary, precursor-free amino acid mixtures (AAM) (AAMs-OAD: amino acid mixtures in OAD) can be supplemented without inducing intoxication, there has recently been some debate on their use with respect to the relative high L-leucine content influencing other plasma BCAA levels.[3–6] In some patients, single amino acids (SAA) can be supplied to fine-tune dietary treatment, though there is no international consensus on the use of either AAMs-OAD or SAA.[7] In UCD patients, the purpose of dietary treatment is to reduce the nitrogen load. In the most severe cases, treatment consists of a protein-restricted diet (either with or without the use of AAM-UCD [amino acid mixture in UCD]), supplemented with L-arginine and/or L-citrulline to support the urea-cycle.[8] Most patients also receive nitrogen scavengers. In patients with mild phenotypes, treatment can be less strict, ranging from a self-initiated vegetarian diet to a natural protein restriction without nitrogen scavengers and other supplements. In both OAD and UCD, additional caloric intake to maintain anabolism is necessary.[9]

In daily practice, dietary treatment is currently very diverse.[4,10] If long-term outcome in these patients is to be improved, it is essential that treatment is optimized. The guideline proposed by Baumgartner et al for the dietary treatment of patients with MMA and PA[11] suggest that "the FAO/WHO/UNU (2007) safe levels of protein intake are a useful guide for protein prescription." The guideline suggests that "synthetic protein should form part of the total protein intake if natural protein tolerance is below FAO/WHO/UNU (2007) safe levels,"[11] and the Genetic Metabolic Dietitian International guideline on PA recommends that AAMs-OAD (which lack L-isoleucine and L-valine) should be added to achieve 100% to 120% of recommended daily allowance (RDA) in those patients who tolerate a natural protein intake less than 100% RDA (Southeast Regional Newborn Screening and Genetics Collaborative [SERC][12]). However, there is no guideline available on the amount of synthetic protein intake as percentage of total protein intake in those patients requiring supplemental amino acids. The guideline for UCD proposed by Häberle et al[13] likewise suggests that the FAO/WHO/UNU requirements are used for protein intake. The guideline provides recommendations on the amount of synthetic protein intake as a percentage of the total protein intake for patients who require supplemental amino acids (recommendation: 20%-30%). The same guideline also propose recommendations on dosages of L-citrulline and/or L-arginine treatment (L-arginine dose: <20 kg body weight: 100-200 mg/kg/d, >20 kg body weight: 2.5-5 g/m^2/d, and L-citrulline dose: 100-200 mg/kg/d). However, these dietary and supplemental recommendations are based on expert opinion. There are neither outcome data with respect to recommendations on appropriate plasma amino acid levels, nor are there clear recommendations on whether L-citrulline or L-arginine treatment is preferred in any of the following: CPS1-D, OTC-D, and HHH syndrome.

If optimized treatment is feasible, it is likely to improve outcome in OAD and UCD patients. To establish whether or

not treatment is sufficient, it is very important to evaluate dietary and supplemental treatment in a large cohort of OAD and UCD patients. We evaluated data from the first visit recorded in the registry from OAD and UCD patients in the European registry and network for Intoxication type Metabolic Diseases (E-IMD) (a) to compare their current long-term dietary and supplemental treatment with the existing guideline; and (b) to study the plasma amino acids levels in patients with this prescribed treatment.

2 | METHODS

2.1 | Patient registry and inclusion/exclusion criteria

The European registry and network for Intoxication type Metabolic Diseases (E-IMD; URLs: http://www.e-imd.org [website]; https://www.eimd-registry.org [registry]) is a European project that was initiated in 2011 and now includes a web-based patient registry containing comprehensive follow-up data on more than 1200 individuals with OAD and UCD. A detailed overview of the E-IMD was published previously.[14] The data in the E-IMD registry were entered by clinicians and dieticians. The dietary information in this registry was the diet prescribed, which does not necessarily equal the consumed diet. The study was approved by the local ethics committee of the coordinating center (University Hospital Heidelberg) and by all clinical partners. The current publication project was evaluated by the scientific board and approved by the executive board of the E-IMD consortium. All the procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national) and with the Helsinki Declaration of 1975 as revised in 2000. Informed consent with regard to being included in the study was obtained from all patients or their legal guardians prior to being included in the study in countries where this was needed by law.

In this cross-sectional study, we included all data from the first visits recorded in the registry and all visits included were during stable metabolic period. We included MMA (mut-, mut0, cblA, and cblB), PA, OTC-D females and males, CPS1-D, HHH syndrome, ASS-D, and ASL-D patients. CblA and CblB patients were included as the majority of them were symptomatic and they were also treated with a natural protein restriction. We excluded patients with other inherited metabolic diseases, those with an unconfirmed suspicion of an OAD or UCD, those with MMA CblC and CblD subtypes and unclassified type, and those who had received a kidney or liver transplantation or had other serious unrelated comorbidities, such as Down syndrome, kernicterus, or fetal alcohol syndrome. We furthermore excluded those without information on the prescribed dietary treatment and those without information on clinical symptoms (symptomatic or not). Clinically symptomatic patients were defined as presenting with a metabolic crisis or long-term complications.

2.2 | Data analysis

Synthetic protein was defined as a protein-equivalent of specialized AAM-OAD/UCD and SAA. L-valine and/or L-isoleucine were supplied independently or in combination as SAA. Total protein prescription was calculated by adding natural protein and synthetic protein prescription. Protein prescription (natural, synthetic, and total) data from the first visit recorded in the registry was compared with the WHO RDA[15] (Geneva, Switzerland). Synthetic vs total protein prescription (ratio) was calculated by dividing the amount of synthetic protein % RDA by total protein % RDA. Information on the amino-acid content of AAMs-OAD/UCD was obtained from the manufacturers. Supplementation with L-arginine and/or L-citrulline and the provision of sodium phenylbutyrate were both compared with current recommendations.[13] Plasma amino acid levels were compared with the amino acid reference values provided in Table 2.1.5 of the "Laboratory Guide to the Methods in Biochemical Genetics" by Duran.[16]

On the basis of their common metabolic amino acid disturbances, we combined the following disorders in several analyses: (a) MMA and PA; (b) inherited deficiency of ASS-D, ASL-D; (c) OTC-D males, CPS1-D, and HHH syndrome, and (d) as a separate group OTC-D females.

2.3 | Statistical analysis

SPSS (IBM SPSS Statistics 24.0, IBM Corp., Armonk, NY, USA) was used for descriptive statistics (percentages, mean, SD, median, and range). Normality was examined using the Kolmogorov-Smirnov test and quantile-quantile (Q-Q) plots. We corrected the *P*-values per outcome for the subgroup analyses by using the Holm method. Student's *t* test was performed to compare means if distribution was Gaussian and Wilcoxon rank sum test (Ws) if distribution was nonnormal. If there were dichotomous parameters in a small sample size Fisher's exact test was performed to determine statistical significance between groups and if there were dichotomous parameters in a large sample size chi-square test was performed. One-way analysis of variance was performed to compare more than two groups. To compare L-citrulline and L-arginine prescription against the guideline, body-surface area was calculated with the Mosteller formula. To identify variables that had significant associations with plasma amino acid levels, we used backward stepwise linear regression

analysis to explain the relationship between five independent variables on plasma amino acids. As forward methods produce suppressor effects, we performed backward linear regression.[17] Five independent variables were used in the multiple regression analysis: protein prescription (natural [% RDA] and synthetic); use of single BCAAs (yes/no); age at visit; clinically symptomatic (yes/no); and, in UCD, use of sodium phenylbutyrate (yes/no). On the basis of the amount of L-arginine/L-citrulline prescription and of selective L-citrulline vs L-arginine supplementation, we also used backward stepwise linear regression to identify factors that had an association with plasma L-arginine levels in OTC-D and CPS1-D and HHH syndrome.

3 | RESULTS

3.1 | Description of the study population

Two hundred and seventy-one MMA and PA patients and 361 UCD patients had been registered between 1 February 2011 and 20 May 2016 (Supporting Information, Tables S1 and S4). In patients with MMA and PA as well as in patients with UCD, OTC-D females excluded, 82% was symptomatic. A total of 67% of females with OTC-D were symptomatic. In total, 73% of patients with OAD and UCD were diagnosed by selective metabolic testing, 13% by newborn screening, 13% by high-risk family or population screening, and 1% by prenatal testing. Most patients were from Europe (95%), the remainder from Taiwan (2%), the United States (2%), India (0.8%), and Japan (0.2%). Median age at first visit recorded in the registry was 9.3 years (5%-95% percentile: 0.4-35.6). The median time from diagnosis until the first visit recorded in the registry was 6.6 years (5%-95% percentile: 0.2-23.4). Information on participating countries and disease groups is provided in Table S2.

3.2 | MMA and PA

3.2.1 | MMA, PA, and protein prescription

A total of 92% (250/271) MMA, PA patients received a protein restricted diet (Figure S1). Natural protein prescription was according to and above the RDA in 62% (155/250). Symptomatic MMA, PA patients received a lower natural and total protein prescription % RDA than asymptomatic patients (Table S3, Figure 1A). A few patients were prescribed a natural protein intake <50% or >200% RDA (Figure 1A). Various (29) AAMs-OAD were provided by a total of six companies and used for a majority of MMA and PA patients (Figure S1). AAMs-OAD were free of L-valine and L-isoleucine, while the rest of their content varied (Table S4). Median protein prescription through AAMs-OAD was 0.50 g/kg/d (5-95% percentile: 0.18-1.20). SAA were supplied in only 17% of those with MMA (24/144) and 20% of those with PA (25/127). Ninety percent of those who received SAA (44/49) were also prescribed AAM-OAD. In patients who received synthetic protein, natural protein prescription was according to and above the RDA in the majority (58%, 96/166) (Figure 2, Table S3). The mean amounts of synthetic protein as a percentage of total protein prescription was 40% (SD ±15.2). In patients with MMA, those with the Mut^0 subtype were prescribed the lowest natural protein % RDA. AAMs-OAD were most frequently used in Mut0 and CblB patients (Table S5).

Protein prescription within the different countries varied, with a median natural protein prescription of 112% RDA, a total protein prescription of 159% RDA and a synthetic vs total protein prescription of 40% (Table S2). A high natural protein prescription was seen in the Austria (n = 12), Taiwan (n = 7), and Romania (n = 2) and the highest total protein prescription in Czech Republic (n = 10), Spain (n = 20), Romania (n = 2), the United States (n = 10), and Japan (n = 1). The synthetic vs total protein prescription was highest in United Kingdom (n = 1), Japan (n = 1), Denmark (n = 4), Spain (n = 13), and Portugal (n = 2) (Table S2). Compared to the other countries, AAM-OAD was not applied in Serbia and Taiwan.

3.2.2 | Impact of dietary management on plasma amino acids in MMA, PA

Plasma BCAA levels were reported in 66% (180/271) of the MMA and PA patients (Table S1). In individuals with MMA and PA plasma, L-valine and L-isoleucine levels both lay below the lowest reference ranges in 57% and 55% of the patients, respectively (Figure 3A). Symptomatic MMA and PA patients who were prescribed AAM-OAD had lower plasma L-valine and L-isoleucine levels than those without (Figure 4, Table S3). Linear regression analysis showed that the plasma L-valine was positively associated with the amount of natural protein prescription % RDA and negatively associated with the amount of L-leucine prescription derived from AAM-OAD (Table 3c). Plasma amino acid levels of L-isoleucine, L-valine, and L-leucine were the lowest in patients with Mut0 and cblB phenotype (Table S5). In MMA and PA median plasma, L-isoleucine:L-leucine:L-valine ratio was 1:2.5:2.9 (reference value: 1:2: 4). Patients with AAM-OAD had ratio of 1:3.0:3.2, while those without AAM-OAD had ratio of 1:1.9:3.3. The ratio of L-leucine vs L-isoleucine plasma levels ($Ws = 4088.0$, $Z = -4.590$, $P < 0.001$) as well as the ratio of L-leucine vs L-valine plasma levels ($Ws = 3569.5$, $Z = -6.084$, $P < 0.001$) were significantly higher in patients who received AAM-OAD compared to those without.

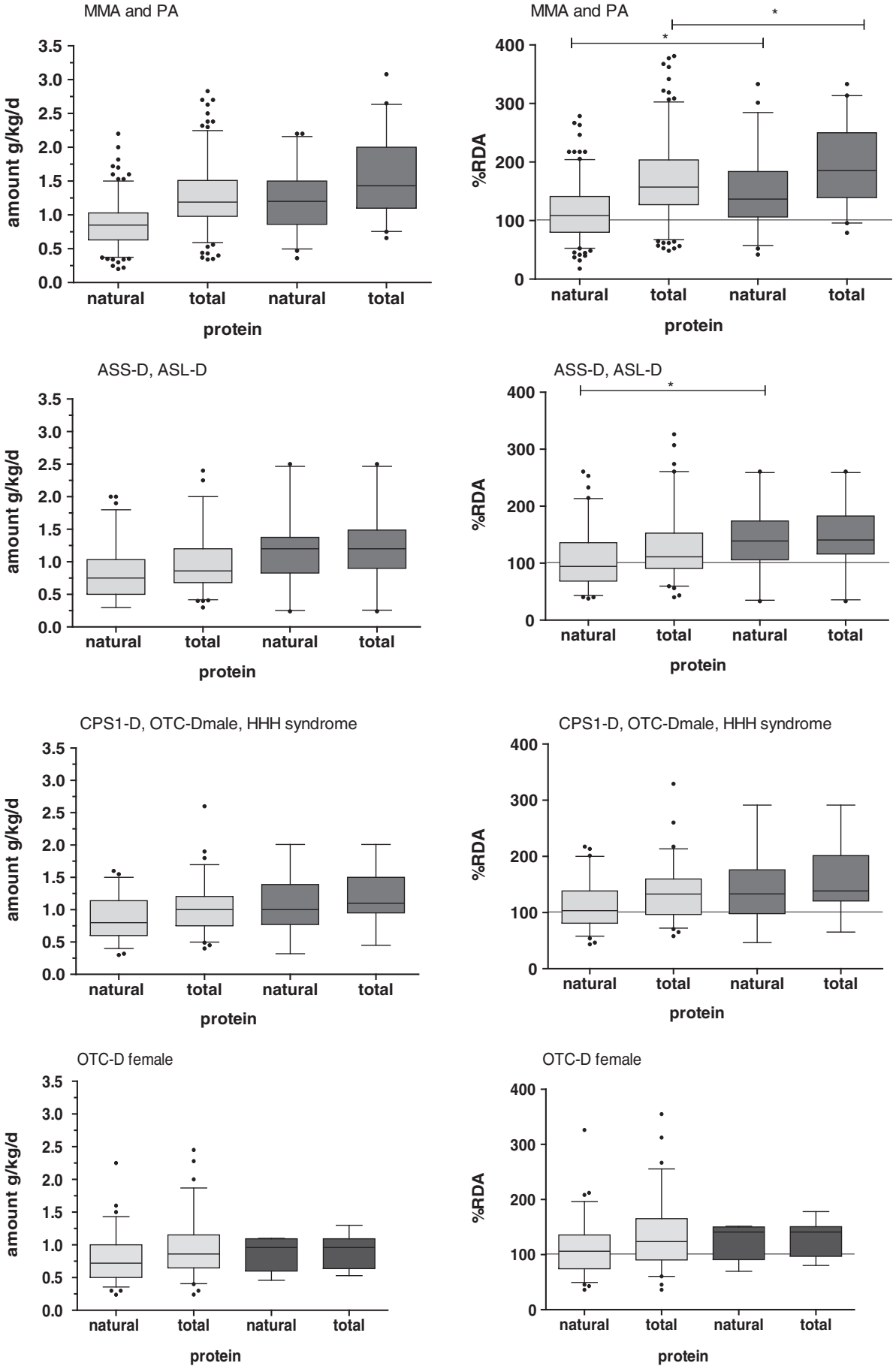

FIGURE 1 Legend on next page.

3.3 | Urea-cycle disorders

3.3.1 | UCD and protein prescription

A total of 80% (289/361) of the UCD patients received a protein-restricted diet (Figure 1). Median natural protein prescription in symptomatic and asymptomatic patients was close to or above the RDA; in the majority of UCD subgroups, total protein prescription was above the RDA (Figure 1A-D). Several patients were prescribed a natural protein intake protein <50% or >200% RDA (Figure 1B-D). For UCD 21 different AAMs-UCD were provided by a total of five companies. AAMs-UCD were supplemented with BCAA (L-valine, L-isoleucine, and L-leucine) (Table S4). AAMs-UCD were used for 32% (114/361) of the patients (Figure 1) and median protein prescription through AAM-UCD was 0.28 g/kg/d (range: 0.04-1.17 g/kg/d).

Symptomatic patients who received AAM-UCD had a lower natural protein % RDA prescribed than those who did not receive AAM-UCD in the CPS1-D, OTC-D male, and HHH-syndrome subgroup (Table 3b). In the ASS-D, ASL-D and CPS1-D, OTC-D male and HHH-syndrome subgroups, natural protein prescription in symptomatic patients taking AAM-UCD lay below the RDA (Figure 2). SAA of BCAA were supplied in only 3% (11/361); 3/11 of those who received SAA were also prescribed AAM-UCD. The mean amounts of synthetic protein as a percentage of total protein prescription were as follows in the following groups: 31% in the ASS-D and ASL-D subgroup (SD ±18.2), 32% in the CPS1-D, OTCD-male, and HHH subgroup (SD ±11.7) and 28% (SD ±14.2) in OTC-D females.

Protein prescription within the different countries varied, there was a high median natural protein prescription in Poland (n = 9), Denmark (n = 10), and India (n = 4), while a low median natural protein prescription in Austria (n = 3), Italy (n = 39), and United Kingdom (n = 8). Austria and the United Kingdom had a relatively high synthetic vs total protein prescription and thereby a total protein prescription according to recommendations, while Italy had a low total protein prescription. AAMs-UCD were prescribed in the majority of patients in the Netherlands, Austria and Greece, Czech Republic, while in the minority in all other countries (Table S2).

3.3.2 | Sodium phenylbutyrate in UCD

Sodium phenylbutyrate was provided in 37% of the UCD patients (134/361). In the CPS1-D, OTC-D male and HHH-syndrome subgroup, prescribed natural protein and total protein % RDA was lower in those prescribed sodium phenylbutyrate treatment than in those who were not (Table S3B). Sodium phenylbutyrate was applied in the majority of patients in Germany, the Netherlands, Croatia, Czech Republic, and Taiwan (Table S2D).

3.3.3 | Impact of dietary management and sodium phenylbutyrate on BCAAs in UCD

In 76% of UCD patients (277/361), BCAA levels were reported (Table S1). In total UCD, plasma L-valine, L-isoleucine, and L-leucine levels lay below the levels of reference values in 18%, 30%, and 31% of the patients, respectively (Figure 3B-D). Table S3B shows plasma BCAA levels of the ASS-D, ASL-D subgroup, OTC-D male, CPS1-D, HHH syndrome subgroup, and OTC-D female subgroup in symptomatic vs asymptomatic patients and in those with a natural protein restricted diet vs those without. In these UCD subgroups, plasma BCAA levels in patients who received AAM-UCD did not differ from those who did not receive AAM-UCD (Table S3B). In total UCD patients, linear regression analysis showed that plasma L-valine, L-isoleucine, and L-leucine were associated with sodium phenylbutyrate treatment and age at visit (Table S3D). The UCD patients had a plasma L-isoleucine:L-leucine:L-valine ratio of 1:1.7:3.7 (reference value: 1:2:4). Patients who received AAM-UCD had a ratio of 1:1.7:3.7 and those who did not receive AAM-UCD had ratio of 1:1.9:3.7.

3.3.4 | L-arginine and/or L-citrulline treatment in UCD

L-arginine was provided in most ASS-D and ASL-D patients, that is, in 95% of the symptomatic patients (90/95) and 86% of the asymptomatic patients (18/21). In 31% of ASS-D (18/58) and 31% of the ASL-D patients (14/45), L-arginine-supplemented doses were above the maximum recommended guideline (6 g/d[13]). Individuals who received L-arginine in the ASS-D and ASL-D subgroup had lower natural protein % RDA and total protein % RDA prescription than those who did not receive L-arginine treatment (Table S3B).

In the CPS1-D, OTC-D male and HHH-syndrome subgroup, 86% of the symptomatic patients (82/95) and 59% of the asymptomatic patients (13/22) were prescribed L-citrulline and/or L-arginine. One or both of these amino

FIGURE 1 Protein prescription in patients with methylmalonic aciduria (MMA) and propionic aciduria (PA) (A); patients with argininosuccinate synthetase deficiency (ASS-D) or argininosuccinate lyase deficiency (ASL-D) (B); patients with carbamylphosphate synthetase 1 deficiency (CPS1-D), males with ornithine transcarbamylase deficiency (OTC-D) or patients with hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome (C); and in females with OTC-D (D). Gray boxes indicate symptomatic patients and black boxes indicate asymptomatic patients. Synthetic protein indicates the amounts only for patients receiving synthetic protein. Circles: outliers; whiskers: 5 to 95 percentile; horizontal line: median; $*P < 0.05$

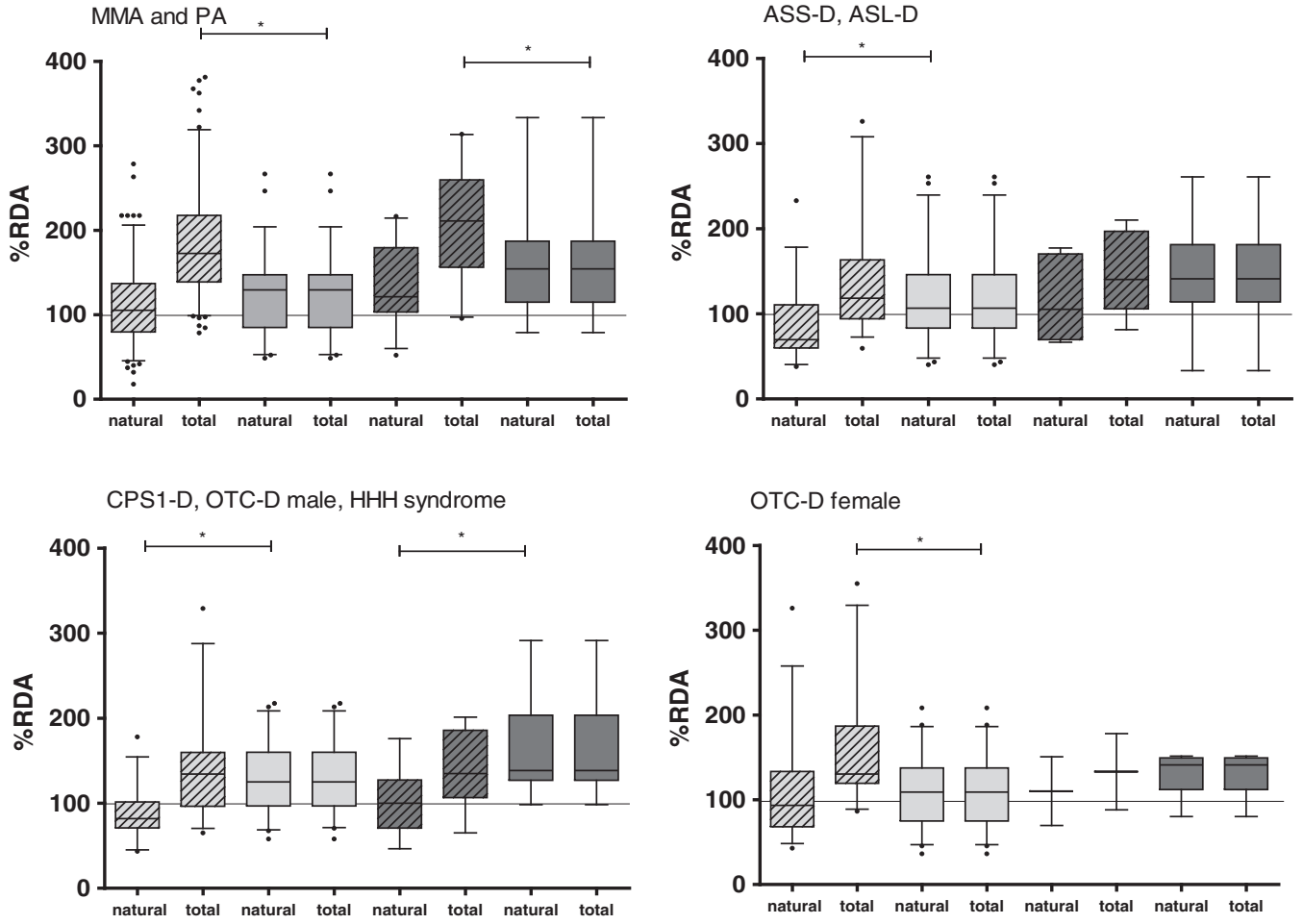

FIGURE 2 Protein prescription and the use of amino acid mixture (AAM). Gray boxes indicate symptomatic patients and black boxes indicate asymptomatic patients. Diagonally striped boxes indicate patients in whom AAM was prescribed, and boxes without stripes indicate patients without AAM. Circles: outliers; whiskers: 5 to 95 percentile; horizontal line: median; $*P < 0.05$

acids were also prescribed in 79% of the symptomatic females with OTC-D (68/86) and in 21% of the asymptomatic females (9/42). In those who received L-citrulline and/or L-arginine, selective supplementation with L-citrulline was provided in 44% (76/172); a combination of L-citrulline and L-arginine was provided in 20% (34/172); and selective L-arginine supplementation was provided in 36% (62/172). Many patients were prescribed L-citrulline (44% [48/109]) and L-arginine (24% [22/92]) above recommended dose.[13]

3.3.5 | UCD and levels of plasma L-arginine and L-citrulline

In the subgroup of patients with CPS1-D, OTC-D [male and female] and HHH syndrome, patients who were prescribed selective L-citrulline supplementation had higher plasma L-arginine levels than those who were prescribed only L-arginine (Ws = 2745.5, Z = −3.066, $P = 0.002$) and they had higher plasma L-arginine levels than those without supplementation (Ws = 1406.0, Z = −5.109, $P < 0.001$) (Figure S2). Plasma L-arginine levels in patients who received L-citrulline only did not differ from those in patients who received a combination of L-citrulline and L-arginine (Figure S2). The difference in plasma L-arginine levels between symptomatic and asymptomatic patients can be found in Table S3B. Plasma L-arginine levels did not differ between OTC-D males and females. The ratio of OTC-D male vs female did not differ within patients receiving either L-citrulline or L-arginine or a combination of these two. Patients (CPS1-D, OTC-D [male and female] and HHH syndrome) who received either L-citrulline, L-arginine, or a combination of both did not differ with regard to the age at visit, prescription of natural protein % RDA, and prescription of protein derived from AAM-UCD. However, the mean L-arginine prescription (mg/kg/d) in patients who received L-arginine was lower than the mean L-citrulline prescription (mg/kg/d) in patients who received L-citrulline (Ws = 3397.0, Z = −2.518, $P = 0.012$). Regression analysis showed that

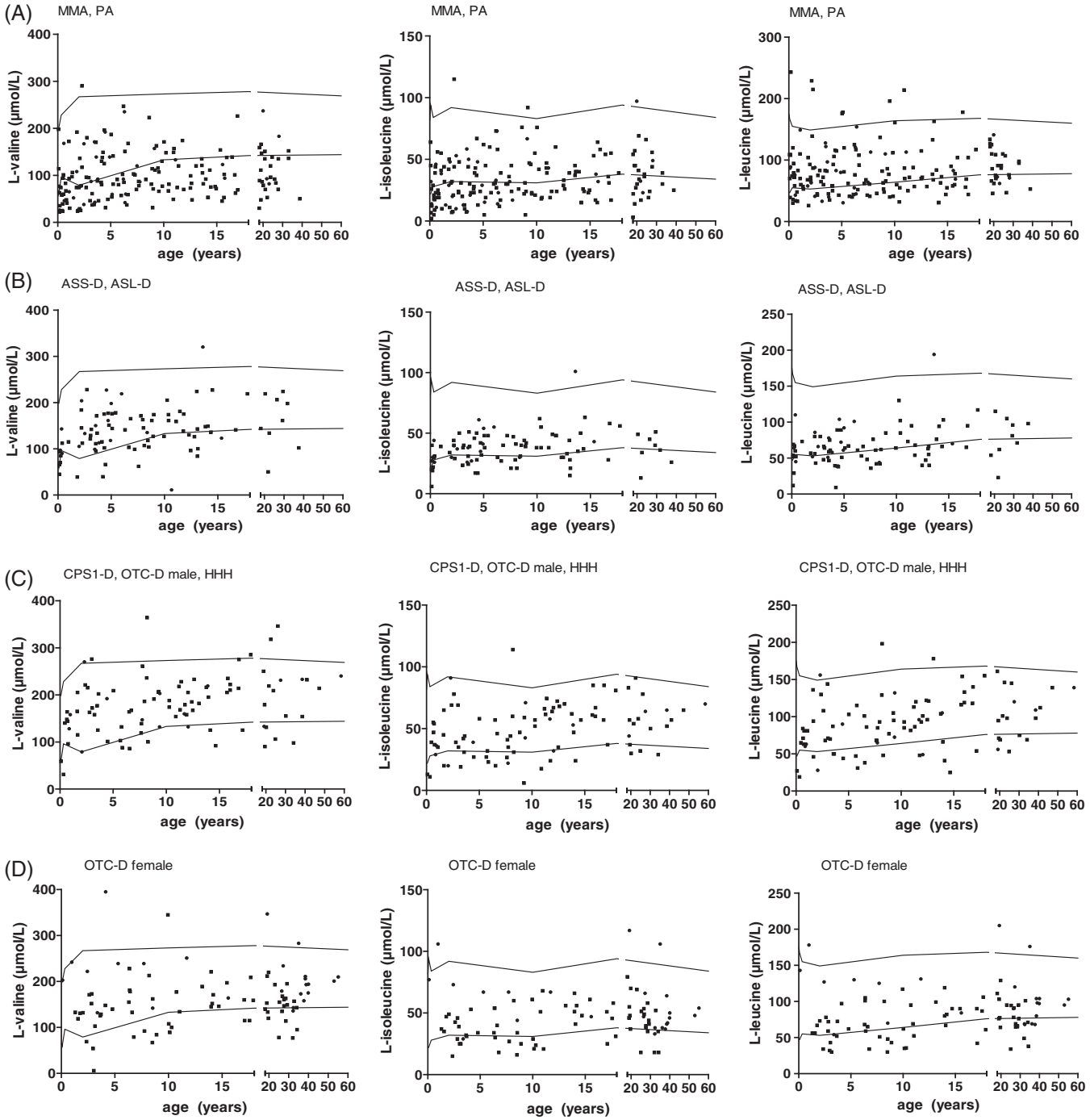

FIGURE 3 Plasma L-valine, L-isoleucine, L-leucine levels in patients with methylmalonic aciduria (MMA) and propionic aciduria (PA) (A); patients with argininosuccinate synthetase deficiency (ASS-D) or argininosuccinate lyase deficiency (ASL-D) (B); patients with carbamylphosphate synthetase 1 deficiency (CPS1-D), males with ornithine transcarbamylase deficiency (OTC-D) or patients with hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome (C); and in females with OTC-D (D). Horizontal lines indicate upper and lower levels of reference values.[16] Each point represents a single measurement for a particular patient

plasma L-arginine levels were associated with selective L-citrulline therapy vs L-arginine monotherapy (β-coefficient = 0.250, $P = 0.007$) ($R^2 = 0.062$, $R^2_{\text{Adjusted}} = 0.054$), while the dose of either L-arginine or L-citrulline was not significantly associated with plasma L-arginine levels in the regression analysis.

4 | DISCUSSION

The purpose of this cross-sectional study was to evaluate current prescribed long-term dietary and supplemental treatment in OAD and UCD. We used the E-IMD registry to survey dietary management approaches of 271 OAD and

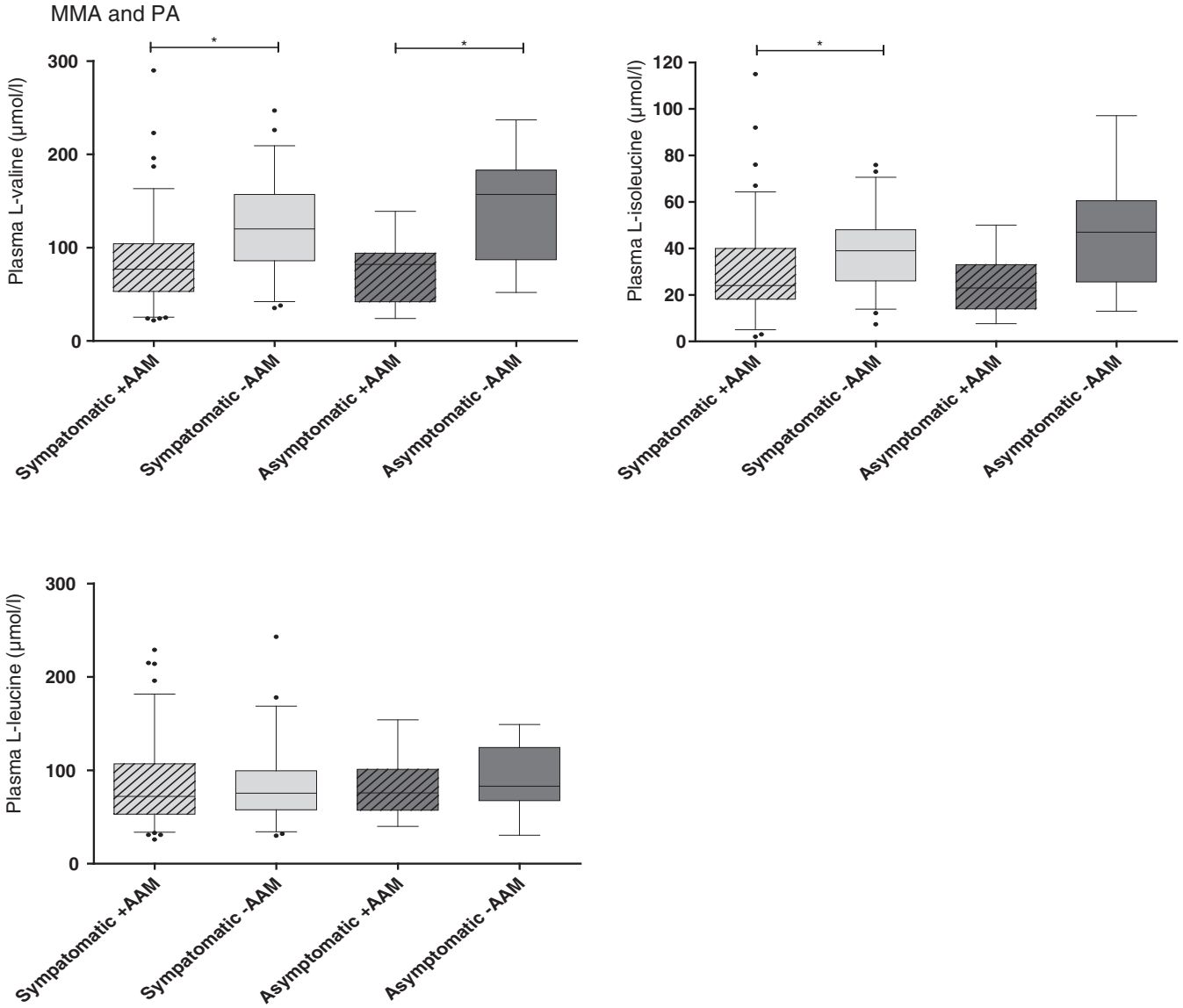

FIGURE 4 Plasma L-valine, L-isoleucine, and L-leucine levels in patients with methylmalonic aciduria (MMA) and propionic aciduria (PA). Gray boxes indicate symptomatic patients and black boxes indicate asymptomatic patients. Diagonally striped boxes indicate patients in whom amino acid mixture (AAM) was prescribed, and boxes without stripes indicate patients without AAM. Reference values are age dependent and can been found in Figure 3A

361 UCD patients to compare their current long-term dietary and supplemental treatment with the existing guideline; and to study the plasma amino acids levels in a total of 457 patients with this prescribed treatment. It is essential to evaluate this treatment, not only because long-term outcome in OAD and UCD continues to be disappointing, but also because newborn screening for these diseases is upcoming in an increasing number of countries.

We have three main findings. First, plasma L-valine and L-isoleucine levels were very low in most patients with MMA and PA, mainly in symptomatic patients who received AAM-OAD (which lack L-valine and L-isoleucine), while median daily natural protein prescription was consistent with the current RDA. The high L-leucine content in AAM-OAD seemed to affect the plasma L-valine levels and lead to abnormal ratios of plasma L-leucine:L-isoleucine and L-leucine:L-valine.

Second, of patients with UCD plasma BCAA levels lay below reference ranges in approximately 20% to 30%. While natural protein prescription lay below the RDA in most symptomatic patients who received AAM-UCD, plasma L-valine and L-isoleucine levels and L-isoleucine:L-leucine:L-valine ratio were similar to those in patients who did not receive AAM-UCD (which contain essential amino acids). Sodium phenylbutyrate was correlated with low BCAA levels as previously reported.[18,19] Third, plasma L-arginine levels were significantly higher in patients with CPS1-D, OTC-D, and HHH syndrome who were prescribed selective

L-citrulline supplementation than in patients who were prescribed no supplementation or selective L-arginine supplementation.

Some limitations of this study and of the registry should be addressed. First, as no diary-based data was available on protein intake and there was no data on the patients' adherence to dietary treatment, we were unable to analyze actual dietary intake and neither total BCAA intake. Furthermore, quality of protein consumed was not known. In this study, we assumed that the patients took all the prescribed amounts. The large number of patients included in this study can give general information. As a next step actual dietary intake and intake of BCAAs and its effect should be evaluated. Second, in this study kilocalorie intake was not incorporated and should be further studied in upcoming studies. Third, there was a high variability in the AAMs-OAD/UCD prescribed and they should be studied in more detail to specifically determine their effects. Fourth, the quantitative amino acid analyses were only a single measurement and not all patients included had BCAA levels measured. Our cross-sectional approach did not allow easy identification of predictors of plasma amino acid levels, it is difficult to assign a cause-effect relationship. Fifth, due to the high number of participating centers plasma amino acids levels are determined by various methods and different reference values are used, which can have impact on the conclusions drawn. No quality checks were in place to ensure the accuracy of data collected at each site. We did not assess the correctness of measurements and the differences between contributing laboratories. However, most laboratories participate regularly in external quality assessment (ERNDIM). Furthermore, the registry does not record the interval between the last intake and sampling of amino-acid plasma levels. However, as compartmentalization of plasma amino acids can affect the interpretation of plasma amino acid levels, samples should be taken accurately between 3.5 and 4 hours after the last meal.[20] Last, there can be a bias in the patients included, since some countries have high numbers of OAD and UCD, but this is not reflected in the E-IMD registry. All the limitations specified here require attention in future studies.

4.1 | MMA, PA: Protein prescription, AAM-OAD, and plasma amino acid levels

Our results show that while natural protein prescription was often close to the RDA and total protein prescription was even above the RDA, with current dietary prescription the majority of patients had plasma BCAA concentrations below the reference ranges. The low BCAA levels in MMA and PA were previously reported by others[4,5,21,22] as well as the observation that daily natural protein prescription was according to the current RDA in MMA and PA.[23,24] Toward improving patient care, it thus seems necessary to fine-tune plasma BCAA levels in individual patients. BCAAs are essential for keeping up anabolism and a decrease in plasma BCAA concentrations can herald an acute metabolic crisis.[18] Furthermore, BCAAs are essential for supporting normal growth and development.[25–27]

Since a low natural protein intake can be potentially harmful, that is, low BCAA levels, one should ensure that each patient achieves a higher natural protein intake in a way that does not cause metabolic instability. Protein prescription is currently based on the recommendations provided by the WHO 2007[15] (Geneva, Switzerland). While these recommendations are based on individuals consuming protein of a high biological value, the proteins commonly used by OAD patients are not only of low biological value, but are also poorly digestible.[24] Consequently, the WHO 2007 recommendation does not seem applicable to these patients, an appropriate guideline for their protein prescription is necessary. However, due to interpatient variability this may be hard to achieve.

In OAD, AAM products are free of L-isoleucine, L-methionine, L-threonine, and L-valine, since these amino acids are precursors of toxic metabolites. While the guideline suggests prescribing AAM-OAD in those patients with a natural protein prescription below the RDA, we observed that the majority of those who received AAM-OAD had natural protein prescription that was according to and above the RDA. Nevertheless, our results show significantly lower plasma L-valine and L-isoleucine levels in symptomatic patients who received AAM-OAD. Those who received AAM-OAD are; in view of the very low L-valine and L-isoleucine levels, likely to be at risk of decompensations and growth retardation, and potentially of long-term complications. It is noteworthy that, in a small cohort of MMA patients, a high intake of L-leucine derived from AAM-OAD was associated with lower L-valine and L-isoleucine plasma levels.[5] This could possibly be explained by means of the competitive interaction of BCAA on the same receptors (such as the large neutral amino acid transporter, LAT1[28]). We confirm this inverse relationship between L-leucine intake derived from AAM-OAD and plasma L-valine levels in our large patient cohort (of MMA and PA patients) with correction for covariates such as natural protein prescription. We furthermore showed that L-leucine:L-isoleucine as well as L-leucine:L-valine ratios were higher in those who received AAM-OAD vs those who did not, which is in line with a study by Myles et al.[6] In patients whose natural protein intake cannot be raised, L-valine and L-isoleucine plasma levels may be optimized by reducing the L-leucine content of AAM-OAD. However, as the coefficients of the negative correlation between L-leucine derived

from AAM-OAD and plasma L-valine and L-isoleucine levels were small, it is possible that such a reduction will be effective only in a small number of patients. It may also be hazardous: although our data do not confirm this, further reducing the daily L-leucine intake might lead to even lower L-leucine plasma levels than found (low plasma L-leucine levels were previously reported[22,23,29]), and thereby increase the risk of catabolism. In conclusion, AAMs-OAD for MMA and PA patients should be prescribed with care and with full awareness of their potentially harmful consequences.[6]

In MMA and PA patients, supplementation with SAA can play a role in dietary treatment.[7] In the overall group of MMA, PA patients, the L-isoleucine:L-leucine:L-valine ratio was 1:2.5:3.0 and in those patients without AAM-OAD ratio was 1:1.9:3.3, which indicates low L-valine levels (normal L-isoleucine:L-leucine:L-valine ratio is 1:2:4[16,28]). The disturbed BCAA ratios in patients without AAM-OAD could be due to the low biological value of protein consumed. In patients where maximal protein tolerance has been reached, SAA might be an option. In our opinion, calculating BCAA ratios can give an indication whether or not to increase natural protein or to supply SAA.

The first step toward better monitoring of OAD patients was provided by the guideline that recommend monitoring quantitative amino acids every 3 to 6 months.[11] As it is very difficult to obtain optimal plasma BCAA levels that stimulate growth and development without inducing toxicity, we suggest that individualized patient care might be optimized by more frequent monitoring of BCAAs plasma levels.

4.2 | UCD: Protein prescription, AAM-UCD, and plasma amino acid levels

Natural protein prescription was often close to the RDA and total protein prescription was even above the RDA in UCD patients. This could be due to the fact that in this study we looked into protein prescription, which is not necessarily equal to the intake. Interestingly, several patients were prescribed high natural protein intake and total protein intake (>200% RDA), which is highly important to be aware of since this puts the patients at risk for hyperammonemia and renal disease. The risk for low plasma BCAA levels is highest in those who received sodium phenylbutyrate.[18,19,30,31] In UCD patients, AAMs-UCD are supplemented with L-valine, L-isoleucine, and L-leucine. We found that the natural protein prescribed in symptomatic UCD patients who received AAM-UCD was lower than recommended, and significantly lower than that in patients without AAM-UCD. Due to AAM-UCD (median dose 0.28 g/kg/d) total protein prescription was consistent with recommendations. We observed that UCD patients who received AAM-UCD—and thus a lower natural protein prescription—achieved plasma L-isoleucine and L-valine levels and L-isoleucine:L-leucine: L-valine ratios similar to patients without AAM-UCD. This suggests that AAMs-UCD have a beneficial effect in UCD in stable disease period.

4.3 | UCD: L-arginine and/or L-citrulline

To date there has been no clear-cut evidence that the efficacy of L-citrulline is greater than that of L-arginine for OTC-D, CPS1-D, and HHH syndrome.[13] Now, for the first time, we show that patients who received L-citrulline had higher plasma L-arginine levels than those who received L-arginine alone. Plasma L-arginine levels depend on the amount of L-citrulline and/or L-arginine prescription. The bioavailability of L-citrulline is greater than that of L-arginine,[32,33] and supplementation with L-citrulline leads to higher plasma L-arginine concentrations than supplementation with L-arginine. This supports the notion that it is preferable to use L-citrulline for patients with CPS1-D, OTC-D, and HHH syndrome. Importantly, L-citrulline is more expensive than L-arginine.[13]

In this study, we surveyed long-term prescribed dietary treatment and amino acid supplementation in OAD and UCD patients in the E-IMD registry, taking the limitations of this study into consideration, with the aim to improve treatment. In future studies the possible harmful consequences (ie, the number of decompensations, growth, long-term complications, and mortality) of the very low plasma BCAA levels in MMA, PA, and UCD patients must be evaluated. Recommendations on adequate plasma levels in OAD and UCD should be formulated and the efficacy of adjusted treatment (including AAM and/or SAA), without inducing toxicity, needs to be followed.

5 | CONCLUSION

Current dietary practice in OAD and UCD patients differ widely. Natural protein prescription was close to the RDA, but very low BCAA levels and abnormal BCAA plasma ratios in patients with MMA and PA who were prescribed AAMs-OAD were observed. UCD patients with a risk of low plasma BCAA levels seemed to benefit from BCAA-supplemented AAMs-UCD. Patients with OTC-D, CPS1-D, and HHH syndrome who received selective L-citrulline supplementation had significantly higher L-arginine plasma levels than patients who received no supplementation or selective L-arginine. These results make it possible to further improve recent treatment recommendations for OAD and UCD.

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CONFLICT OF INTEREST

This research was performed independently of other financial sponsors other than Metakids and Erasmus University Medical Center.

AUTHOR CONTRIBUTIONS

F.M. participated in the study design, data analyses, and data interpretation. She also drafted the manuscript and participated in the pertinent aspects of the planning, conduct, and reporting of the work described in the article. F.G. managed the E-IMD patient registry, participated in the design of the study, data analyses, and contributed to writing and revising the manuscript. P.B. participated in the design of the study. He also contributed to writing and revising the manuscript. A.T.v.d.P. interpreted the data and was responsible for the manuscript revisions. M.L.S. interpreted the data and was responsible for the manuscript revisions. K.A.C. interpreted the data and was responsible for the manuscript revisions. I.B. interpreted the data and was responsible for the manuscript revisions. A.M.L. interpreted the data and was responsible for the manuscript revisions. S.K. coordinated E-IMD, participated in the design and interpretation of the study, and contributed to data interpretation and manuscript revision. M.W. conceived the study, participated in its design and interpretation, acted as principal investigator, and contributed to data interpretation and manuscript revision. She also participated in the pertinent aspects of the planning, conduct, and reporting of the work described in the article. M.W. serves as the guarantor for the article. All authors have read and approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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